Triplex formation with α-L-LNA (α-L-*ribo* configured locked nucleic acid)

Niti Kumar,[†,‡] Katrine E. Nielsen,[†] Souvik Maiti[‡] and Michael Petersen[†,*]

[†]*Nucleic Acid Center, Dept. of Chemistry, University of Southern Denmark, 5230 Odense M, Denmark*
and
[‡]*Structural Biology Unit, Institute of Genomics and Integrative Biology, CSIR, Mall Road, New Delhi 110 007, India*

Supporting Information

EXPERIMENTAL DETAILS

Oligonucleotides. α-L-LNA oligonucleotides were obtained HPLC purified from Exiqon A/S, Copenhagen (www.exiqon.com). DNA oligonucleotides were obtained from DNA Technology, Århus, Denmark.

Buffer. In all experiments the buffer content was 10 mM sodium cacodylate (pH 6.8), 200 mM NaCl and 2 mM $MgCl_2$. The duplex sequence used in the studies was 5'-GCGCGAGAAGAAAAGAAAGCCGG-3':5'-CCGGCTTTCTTTTCTTCTCGCGC-3'.

CD spectroscopy. CD spectra were recorded on a Jasco spectropolarimeter (Japan) equipped with a thermoelectrically controlled cell holder and a cuvette with a path length of 1 cm. The duplex was annealed by heating the samples for 5 min at 90 °C followed by slow cooling (0.5 °C/min) to 10 °C. Subsequently, the third strand was added. The triplex concentrations were 8 μM. For each triplex, scans were recorded from 200 – 350 nm at 10 °C, 40 °C and 70 °C. For each spectrum, two scans were averaged. The spectrum of the buffer was subtracted from each spectrum. CD spectra were collected in units of millidegrees versus wavelength.

UV melting curves. The thermal stability of duplex and triplexes were determined by a PerkinElmer Lambda 35 spectrophotometer equipped with a thermoregulated Peltier element. The duplex was annealed by heating the samples for 5 min at 90 °C followed by slow cooling (0.5 °C/min) to 15 °C. Subsequently, the third strand was added. The triplex concentrations were 1 μM. The melting profile was recorded at 260 nm from 15 to 90 °C at a heating and

cooling rate of 0.5 °C/min. For the determination of the triplex melting temperatures, the duplex melting curve was subtracted from the triplex melting curve and the triplex melting temperature (T_m value) was taken as the midpoint of the resultant curve. To verify melting temperatures, the first derivative of the triplex melting curves was calculated to resolve the two melting domains. The data was analysed to obtain thermodynamic and kinetic parameters by applying the mathematical model of Rougée *et al.* (ref 13 in main text) as described below.

Using a two state model, equilibrium for the triplex to duplex transition can be written as

$$\text{Duplex (D) + Third strand (S)} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \text{Triplex (Tr)} \qquad (1)$$

The rate of Triplex formation is

$$d[Tr] / dt = k_{on} [S] [D] - k_{off} [Tr] \qquad (2)$$

From the experimental absorbance versus temperature curves, the mole fraction of triplex, α, at each temperature is calculated from the heating and cooling curves by

$$\alpha = \Delta \text{ Abs} / (\text{Abs}_{min} - \text{Abs}_{max}) \text{ and } \Delta \text{ Abs} = [\text{Abs}_{(\text{at any temp})} - \text{Abs}_{max}],$$

where $\alpha = 1$ for hybridization to form triplex and $\alpha = 0$ for dissociation of triplex to duplex and the third strand. Substituting α in eq. (2) gives

$$d (\alpha_h) / dt = k_{on} C (1-\alpha_h)^2 - k_{off} (\alpha_h) \qquad (3a)$$

$$d (\alpha_c) / dt = k_{on} C (1-\alpha_c)^2 - k_{off} (\alpha_c) \qquad (3b),$$

where α_h and α_c are the association factors for heating and cooling curves, respectively, C is the molar concentration of duplex (D) and third strand (S) [1 μM, equal stoichometric ratio of duplex and third strand]. Substitution of heating and cooling rate, (dT/dt) in eqs. (3a) and (3b) yield two linear equations with two variables, k_{on} and k_{off},

$$d(\alpha_h)/dT = (dT/dt)^{-1} [k_{on} C (1-\alpha_h)^2 - k_{off} (\alpha_h)] \qquad (4a)$$

$$d(\alpha_c)/dT = -(dT/dt)^{-1} [k_{on} C (1-\alpha_c)^2 - k_{off} (\alpha_c)] \qquad (4b),$$

with (dT/dt) = 0.5 ºC/min = 0.00833 K/s.

k_{on} C and k_{off} were determined at different temperatures, T, near the melting temperature of the triplex. In practice, the calculation was made at each integer value of T, with dα/dT, at a particular temperature T, approximated by $0.5(\alpha_{T+1} - \alpha_{T-1})$. The values of k_{on} C and k_{off} obtained by the method outlined were plotted in Arrhenius plots, ln(k_{on} C) or ln(k_{off}) versus 1/T, giving a positive slope for k_{on} C and a negative slope for k_{off}. Thus, the activation energy, $E_{on,}$ for the association process is negative and activation energy, $E_{off,}$ for dissociation is positive. The enthalpy for triplex formation was determined as $\Delta H^o = E_{on} - E_{off}$ and ΔS^o was determined from the intercepts of the Arrhenius plots. All kinetic and thermodynamic parameters are included tabulated in Table S1.

Electrophoretic mobility shift analysis. The duplex was heated for 5 mins at 90 °C, annealed at room temperature and incubated with the third strand overnight at 4 °C in 10 mM sodium cacodylate buffer (pH 6.8) containing 200 mM NaCl and 2 mM $MgCl_2$. 1 nmol of triplex in each sample was loaded directly on a 15% polyacrylamide nondenaturing gel prepared in 50 mM Tris acetate (pH 7) and 10 mM $MgCl_2$.The gel was run at 4 °C and with a constant voltage of 70 V for 16 hours. Subsequently, the gel was stained in ethidium bromide solution for approximately 15 minutes, rinsed twice with water and visualized using a Typhoon TRIO variable mode phoshorimager.

NMR spectroscopy. NMR experiments were recorded on a Varian Inova 500 spectrometer. The duplex was heated for 5 minutes at 90 °C, annealed at room temperature and incubated with the third strand overnight at 4 °C in a 90% H_2O:10% D_2O solution (pH 6.8) with 200 mM NaCl and 2 mM $MgCl_2$. The triplex concentration for **ON0** was 0.17 mM and for both **ON4** and **ON5** 0.086 mM. 1D Watergate NOESY spectra were recorded for the triplexes formed by **ON0**, **ON4** and **ON5** at 15 and 25 °C. Each spectrum was acquired with ca 29,000 scans.

Table S1. Kinetic and thermodynamic data of triplex formation determined from the heating and cooling UV melting curves at pH 6.8; α-L-LNA nucleotides are shown in bold red.[a]

		T_m (ºC)[b]	k_{off} (10^{-4} s^{-1})[c]	k_{on} (10^4 $M^{-1}s^{-1}$)[c]	ΔHº (kcal/mol)[d]	ΔSº (cal/mol/K)[d]	ΔG°_{288} (kcal/mol)[d]
ON0	CTCTTCTTTTCTTTC	27.0	3.0	2.0	−54	−167	−5.7
ON0^{Me}	**C**T**C**TT**C**TTTT**C**TTTC	33.5	2.9	1.7	−47	−143	−5.9
ON1	**C**T**C**TT**C**T**T**T**C**T**T**C	55.3	0.52	0.98	−83	−251	−10.9
ON2	C**T**C**T**T**C**T**T**T**C**T**T**C	45.5	0.78	0.90	−58	−176	−7.5
ON3	**C**TC**T**TC**T**T**T**C**T**T**C**	47.0	0.75	0.85	−63	−192	−7.5
ON4	C**T**CTT**C**T**T**T**C**T**T**C	57.4	0.45	0.80	−93	−283	−11.9
ON5	CT**C**TT**C**TT**T**TC**T**TTC	53.2	0.62	1.0	−92	−283	−10.7
ON6	**C**TCT**T**CTT**T**TCT**T**TC	47.3	0.72	1.2	−70	−217	−7.6
ON7	C**T**CTT**C**TTT**T**CTT**T**C	51.8	0.65	1.7	−81	−249	−8.9
ON8	CT**C**TTC**T**TTT**C**TTTC	50.6	0.69	0.98	−74	−288	−8.0
ON9	**C**T**C**TT**C**TTTT**C**TTT**C**	41.5	0.85	0.90	−58	−178	−6.9

[a] **C** is 5-methylcytidine. The 15-mer TFOs were targeted to a 23 base pair dsDNA duplex. [b] Estimated error ± 0.5 °C. [c] Estimated error ± 20 %. [d] Estimated error ± 10 %.

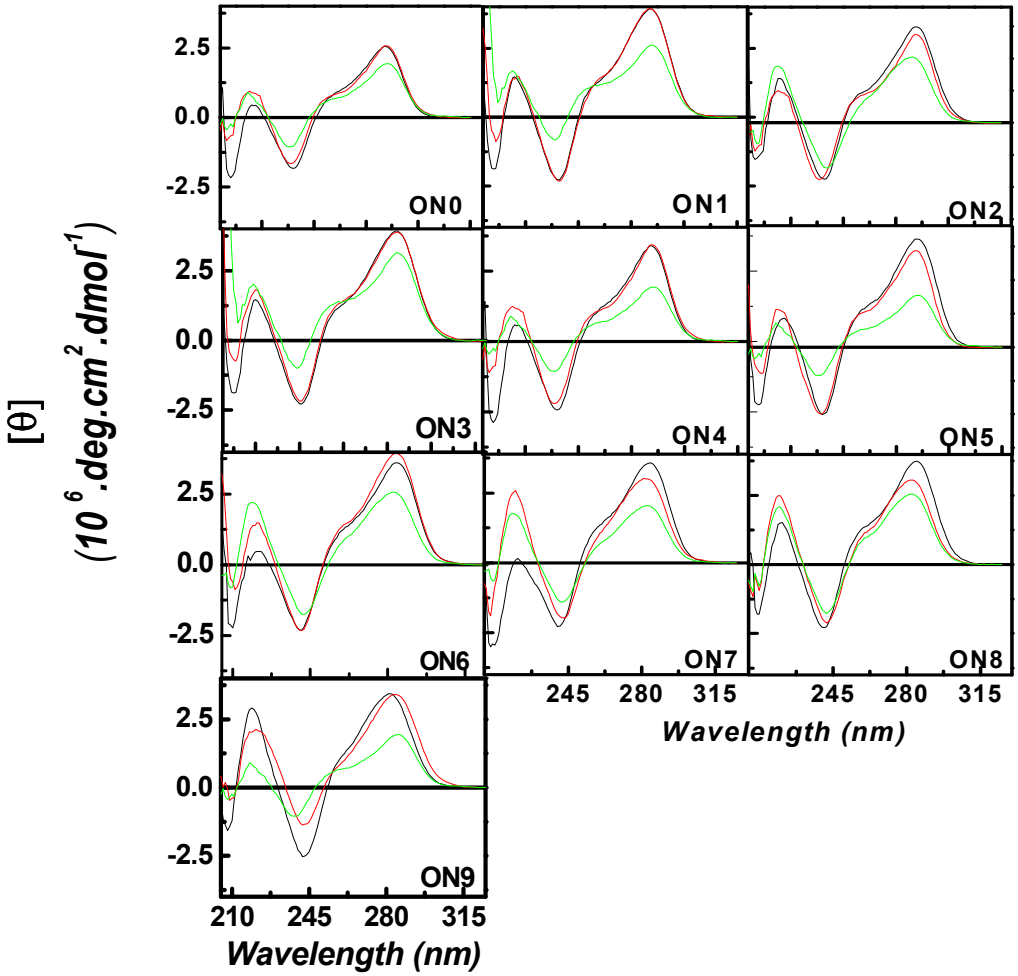

Figure S1. CD spectra of the triplexes formed by TFOs **ON0** to **ON9** at 10 °C (black), 40 °C (red) and 70 °C (green).

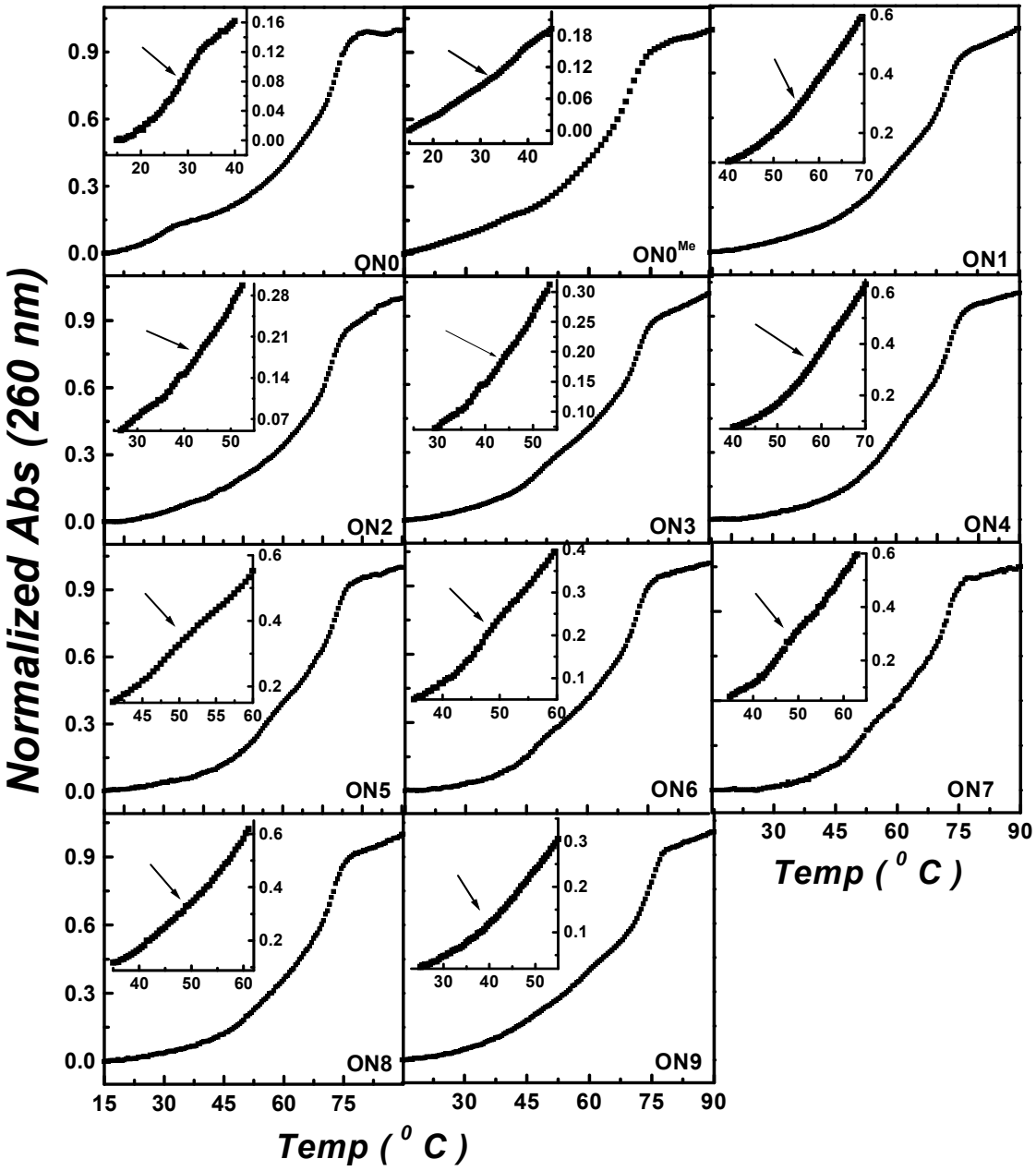

Figure S2. UV melting curves of the triplexes formed by TFOs **ON0** to **ON9**. Normalized absorbance vs temperature recorded at a heating rate of 0.5 °C/min. The inset shows the triplex melting domain of each of the curves.

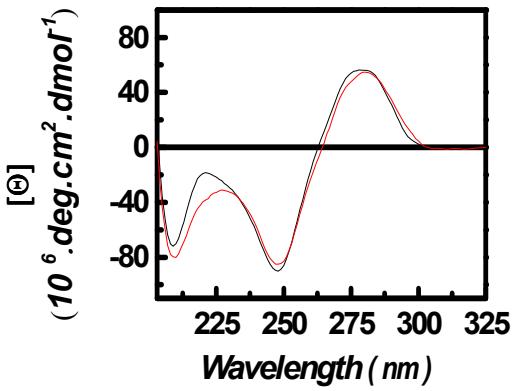

Figure S3. Overlay of the CD spectra of the triplexes formed by TFOs **ON0** (black) and **ON0^{Me}** (red) at 20 ºC.

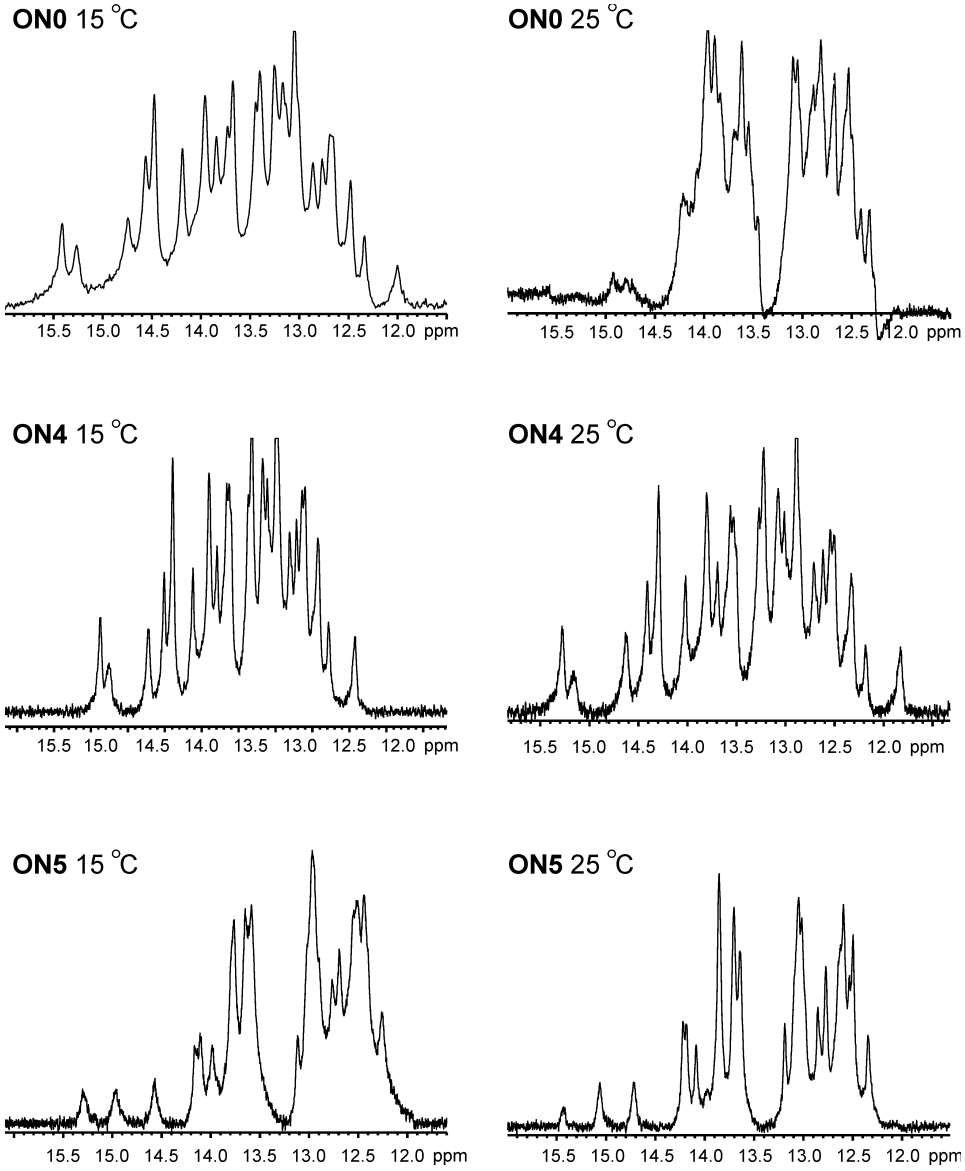

Figure S4. Imino region of 1D ^{1}H NMR spectra of the triplexes formed by TFOs **ON0**, **ON4** and **ON5** recorded at 15 ºC and 25 ºC.